



Addition of mushroom powder to pasta enhances the antioxidant content and modulates the predictive glycaemic response of pasta



Xikun Lu^{a,b}, Margaret A. Brennan^{a,*}, Luca Serventi^a, Jianfu Liu^c, Wenqiang Guan^c, Charles S. Brennan^{a,b,c}

^a Department of Wine, Food and Molecular Biosciences, Lincoln University, P O Box 84, Lincoln 7647, Christchurch, New Zealand

^b Riddet Institute, Palmerston North, New Zealand

^c Tianjin Key Laboratory of Food Biotechnology, College of Biotechnology and Food Sciences, Tianjin University of Commerce, Tianjin, China

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ABSTRACT

This study reports the effects of addition of mushroom powder on the nutritional properties, predictive *in vitro* glycaemic response and antioxidant potential of durum wheat pasta. Addition of the mushroom powder enriched the pasta as a source of protein, and soluble and insoluble dietary fibre compared with durum wheat semolina. Incorporation of mushroom powder significantly decreased the extent of starch degradation and the area under the curve (AUC) of reducing sugars released during digestion, while the total phenolic content and antioxidant capacities of samples increased. A mutual inhibition system between the degree of starch gelatinisation and antioxidant capacity of the pasta samples was observed. These results suggest that mushroom powder could be incorporated into fresh semolina pasta, conferring healthier characteristics, namely lowering the potential glycaemic response and improving antioxidant capacity of the pasta.

1. Introduction

While durum wheat semolina is traditionally used, recent research has focussed on bioactive ingredients to improve physical and nutritional qualities of pasta (Foschia, Peressini, Sensidoni, Brennan, & Brennan, 2015). Much of this interest is related to the manipulation of glycaemic index (GI). GI is a measure of the rate at which carbohydrates in foods are converted to sugar components and how these foods affect postprandial blood glucose responses (Foschia et al., 2015). Clinical research has shown a correlation between low GI diets in individuals with diabetes and the risk of hyperlipidaemia and cardiovascular diseases (Dona, Pages, Gilbert, & Kuchel, 2010). Researchers have proposed that highly digestible (high GI) starchy foods affect satiety and are associated with increased tendency to snack between meals (Dona et al., 2010). Conversely, foods that are considered to be low-GI have been shown to prolonging satiety and improve insulin sensitivity (Chillo, Ranawana, Pratt, & Henry, 2011).

Pasta is an important staple food widely consumed around the world that is considered to be low GI due to the slow rate of starch degradation following ingestion (Foschia et al., 2015). The main component of pasta is starch, and many studies have used dietary fibre and protein to enhance the nutritional quality of pasta. These additional

ingredients have included wheat bran (Sobota, Rzedzicki, Zarzycki, & Kuzawinska, 2015), inulin, β -glucan, guar gum or bamboo fibre (Chillo, Ranawana, & Henry, 2011; Foschia et al., 2015) fish material (Parvathy, Bindu, & Joshy, 2017) and other functional ingredients (Jan, Saxena, & Singh, 2017; Martínez, Marín, Gili, Penci, & Ribotta, 2017). However, to the authors' knowledge, little work has been undertaken regarding substitution of semolina with mushroom powders to produce pasta like products. Generally, mushroom powder is a rich source of protein and dietary fibre compared with semolina and while mushrooms may contain more fat than semolina 75% of this is in the form of polyunsaturated fatty acids (Bach, Helm, Bellettini, Maciel, & Haminiuk, 2017; Ni, Xu, Bu, & Ying, 2017, Rašeta et al., 2016). Additionally, mushrooms contain bioactive components that have been reported to be effective antioxidants, especially the phenolic compounds and polysaccharides (Cheung, 2008, Bach et al., 2017; Li et al., 2017; Wu, Chen, Wang, & Shyu, 2017). products.

Many attempts have been made to mimic digestion *in vitro*, which include studies on the evaluation of glycaemic response to pasta. In this study, the GI of pasta containing 5%, 10% and 15% mushroom powder were evaluated using an *in vitro* model system, as described by Gao, Brennan, Mason, and Brennan (2016).

* Corresponding author.

E-mail addresses: Xikun.Lu@lincolnuni.ac.nz (X. Lu), Margaret.Brennan@lincoln.ac.nz (M.A. Brennan), Luca.Serventi@lincoln.ac.nz (L. Serventi), jianfuliu@aliyun.com (J. Liu), guanwenqiang@tjcu.edu.cn (W. Guan), Charles.Brennan@lincoln.ac.nz (C.S. Brennan).

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2. Materials and methods

2.1. Materials

Commercial semolina (Sun Valley Foods, Auckland, New Zealand), dried shiitake mushroom and porcini mushroom sliced (Jade Phoenix, China) were used in this study, together with fresh white button mushroom obtained from Meadow Mushrooms (Christchurch, New Zealand).

2.2. Pasta preparation

White button mushrooms were cleaned, sliced and then dried at 55 °C for 24 h. In total 1 kg of these dried white button mushroom as well as shiitake and porcini mushroom slices were ground individually into powders using a mixer (model: BCG200, Coffee Grinder, Breville, Australia). The powders were stored in a sealed bag at room temperature until required.

Pasta was produced using a Filmar pasta machine fitted with a 2.25 mm spaghetti die (model: MPF15N235M, Filmar, Villa Verucchion, RN, Italy). Dough blends (500 g durum semolina and 32.5 g water/100 g dry components) were mixed for 20 min in the pasta machine and then extruded. The extruded pasta was stored at –18 °C for *in vitro* digestion analysis (Foschia et al., 2015). Mushroom powder enriched formulations replaced 5 g, 10 g and 15 g/100 g (w/w) semolina, respectively. In total, nine different samples supplemented with mushroom powder were produced, substituting semolina with white dried button, shiitake and porcini mushroom powders, respectively. Control samples were prepared using exclusively durum wheat semolina.

For the determination of Swelling Index (SI) and Water Absorption Index (WAI) frozen pasta (20 g) was defrosted for 10 min at room temperature and cooked for 6 min in boiling tap water (600 mL). The SI and WAI of the pasta was then determined using the methods employed by our previous paper (Lu, Brennan, Serventi, Mason & Brennan, 2016).

2.3. Proximal analysis

Total starch was determined using Megazyme starch analysis kits (Megazyme International Ireland Ltd, Wicklow, Ireland), which apply the AOAC Official Method 996.11 (amyloglucosidase- α -amylase method). Total dietary fibre (TDF) content was determined in duplicate using a total dietary fibre assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland) and measurements were recorded for soluble (SDF) and insoluble fibre (IDF) composition, as described by Brennan, Monro, and Brennan (2008). The protein content was determined using a Rapid Max N exceed (Elementar, Langensfeld, Germany) with conversion factors of 5.7 (AOAC 992.23) and 4.4 (Mariotti, Tome, & Mirand, 2008) for semolina and mushroom powder, respectively. The total fat content was determined using a Soxhlet extraction method.

2.4. Near infrared spectroscopy

Near infrared spectra of cooked pasta samples were determined using Calorie Answer™ (CA-HM, JWP, Japan) according to the method reported by Lau, Goh, Quek, Lim, and Henry (2016). The reflectance mode was used in combination with cereals-powdered cereals and dried noodles modes for analysis. Each triplicate portion was scanned 10 times, and the averaged mean spectrum obtained to improve accuracy by the software (CA-HM Measurement Application Software, JWP, Japan). The primary constituents of foods are C–H, O–H and N–H groups. Fat, carbohydrate and water content can be estimated based on the different energies absorbed at specific wavelengths for these functional groups, which can in turn be used to obtain the metabolisable energy of the samples.

2.5. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (TA Q20, TA Instruments, Newcastle, DE) was used to measure the starch gelatinisation characteristics of raw and cooked pasta samples, using the method reported by Aravind, Sissons, Egan, and Fellows (2012) with some modifications. Ground samples (4–6 mg) were weighed into a Differential Scanning Calorimeter (DSC) pan with 10 μ l of water, which was hermetically sealed and allowed to equilibrate overnight at room temperature. The reference sample was an empty pan and lid which were pressed together. Processing was performed from 10 °C to 110 °C at a rate of 10 °C/min. A DSC heating curve was generated and measurements conducted in duplicate. Temperatures of onset (T_{onset}), gelatinisation (peak), endset (T_{endset}) and the enthalpy of the transition (ΔH) were obtained.

2.6. *In vitro* digestion analysis

Frozen pasta (20 g) was defrosted for 10 min at room temperature and cooked for 6 min in boiling tap water (600 mL). The pasta was drained and cut with a knife to obtain 2–5 mm size pieces (Foschia et al., 2015). An *in vitro* digestion process was used to evaluate sugar release over a period of 120 min, as described previously (Gao et al., 2016).

2.7. Antioxidant analysis

Total phenolic content (TPC) of all samples was measured using 0.2 N Folin-Ciocalteu reagent (Sigma, St Louis, USA) according to the method reported by Singleton and Rossi (1965). The results are expressed as gallic equivalent per gram dry weight. The scavenging capacity of all samples for the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was determined using the method of Floegel, Kim, Chung, Koo, and Chun (2011). The results are expressed as micromoles of Trolox per gram dry weight. The oxygen radical absorbance capacity-fluorescence assay was carried out using the method described by Thaipong et al. (2006) and the results expressed as micromoles of Trolox per gram dry weight.

2.8. Microstructure

The microstructure of both longitudinal surface and transverse cross section of raw pasta samples was evaluated by scanning electron microscope (SEM). The raw pasta samples were freeze-dried prior to analysis. Samples were viewed using 500 \times magnification.

2.9. Statistical analysis

Unless stated elsewhere, experiments were performed in triplicate. Statistical differences were determined by one-way analysis of variance (ANOVA) and Tukey's comparison test ($p < 0.05$). Pearson's correlation was also carried out to analyse significant correlations at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

3. Results and discussion

3.1. Nutritional composition

Previously the role of mushroom material on the physical characteristics of pastas has been investigated by our group (Lu et al., 2016); the current research is an extension of this work and evaluated the nutritional enhancement of pasta in more detail. Table 1 shows the nutritional composition of uncooked semolina and mushroom powder-supplemented pastas as well as the cooked pastas.

Dietary fibre (soluble, insoluble and total dietary fibre), protein and fat were significantly higher in the mushroom powder-supplemented

Table 1

The nutrient components of durum wheat semolina, mushroom powder (a) and cooked pasta (b) samples.

(a)							
Sample	Starch g/100 g (Dry basis)	IDF g/100 g (Dry basis)	SDF g/100 g (Dry basis)	TDF g/100 g (Dry basis)	Protein g/100 g (Dry basis)	Fat g/100 g (Dry basis)	Moisture g/100 g
S	73.11 ± 1.17 ^a	2.36 ± 0.12 ^c	1.59 ± 0.32 ^b	3.95 ± 0.44 ^c	11.32 ± 0.28 ^d	1.17 ± 0.09 ^d	13.95 ± 0.17 ^a
WBM	1.90 ± 0.02 ^b	21.58 ± 2.47 ^b	3.02 ± 0.13 ^a	24.61 ± 2.34 ^b	30.52 ± 0.13 ^a	2.42 ± 0.04 ^b	11.05 ± 0.16 ^b
SM	2.45 ± 0.14 ^b	38.85 ± 1.09 ^a	3.12 ± 0.01 ^a	41.97 ± 1.08 ^a	15.04 ± 0.04 ^c	1.48 ± 0.02 ^c	10.31 ± 1.10 ^b
PM	2.62 ± 0.08 ^b	23.12 ± 1.15 ^b	3.14 ± 0.02 ^a	26.26 ± 1.13 ^b	23.83 ± 0.19 ^b	3.03 ± 0.03 ^a	11.70 ± 0.58 ^b
(b)							
Sample	Total starch g/100 g (dry basis)	IDF g/100 g (dry basis)	SDF g/100 g (dry basis)	TDF g/100 g (dry basis)			
CP	69.95 ± 1.58 ^{ab}	2.10 ± 0.02 ^d	1.07 ± 0.04 ^c	3.17 ± 0.02 ^c			
5% WBP	69.11 ± 0.50 ^{abc}	4.02 ± 0.18 ^{bcd}	2.03 ± 0.08 ^{abc}	6.04 ± 0.11 ^{abcd}			
10% WBP	65.10 ± 1.07 ^{def}	4.75 ± 0.27 ^{abc}	2.14 ± 0.05 ^{ab}	6.89 ± 0.32 ^{abc}			
15% WBP	63.40 ± 1.18 ^{ef}	5.49 ± 0.79 ^{ab}	2.63 ± 0.32 ^a	8.13 ± 1.11 ^a			
5% SP	67.90 ± 1.56 ^{bcd}	3.61 ± 0.44 ^{bcd}	1.55 ± 0.09 ^{4bcde}	5.16 ± 0.54 ^{bcde}			
10% SP	64.05 ± 1.16 ^{ef}	5.39 ± 0.63 ^{ab}	1.77 ± 0.07 ^{3bcde}	7.16 ± 0.56 ^{ab}			
15% SP	62.65 ± 0.26 ^f	6.24 ± 0.088 ^a	1.81 ± 0.07 ^{2bcd}	8.04 ± 0.017 ^a			
5% PP	71.61 ± 0.43 ^a	2.95 ± 0.89 ^{cd}	1.23 ± 0.14 ^{de}	4.18 ± 1.03 ^{de}			
10% PP	68.68 ± 0.25 ^{bc}	3.17 ± 0.75 ^{cd}	1.41 ± 0.10 ^{cde}	4.58 ± 0.85 ^{cde}			
15% PP	66.26 ± 0.94 ^{cde}	4.05 ± 0.19 ^{bcd}	1.98 ± 0.42 ^{abc}	6.03 ± 0.23 ^{abcd}			

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$). Abbreviations: S = semolina; WBM = white button mushroom; SM = shiitake mushroom; PM = porcini mushroom; IDF = insoluble dietary fibre; SDF = soluble dietary fibre; TDF = total dietary fibre.

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$). Abbreviations: CP = control pasta; WBP = white button pasta; SP = shiitake pasta; PP = porcini pasta; IDF = insoluble dietary fibre; SDF = soluble dietary fibre; TDF = total dietary fibre.

pasta (white button, shiitake and porcini mushrooms) compared with control (durum wheat only) semolina pasta (Table 1a). Incorporation of mushroom powder significantly increased soluble, insoluble and total dietary fibre contents of the pasta samples (Table 1b). These results are in agreement with those of Cheung (2008), who reported values between 4.5 and 54.5 % for dietary fibre, 19–35% for protein and less than 10% fat by dry weight in mushrooms. Table 1a illustrates that, even though total fibre content of semolina pasta was much lower than mushroom powder-supplemented pasta, the ratio of soluble dietary fibre to total dietary fibre was much higher than in mushroom powder-supplemented pastas. During cooking, some of the soluble dietary fibre may have dissolved in the cooking water, meaning the ratio of soluble to total dietary fibre in control pasta decreased compared to raw semolina (Table 1b).

Compared with the semolina pasta, the mushroom powder-supplemented pastas contained significantly less starch (Table 1a), apart from the 5% porcini mushroom. There were no significant differences between control semolina only pasta and the 5% white button, 5% shiitake and 10% porcini mushroom-supplemented pastas (Table 1b). Generally, the total starch content of supplemented pastas decreased with increasing mushroom powder content. Furthermore, there was strong negative correlation between starch content and IDF, SDF and TDF ($p \leq 0.001$, Supplementary Table 1). Significant positive correlations (Supplementary Table 1) were observed between fibre content (IDF, SDF and TDF) and both SI and WAI, except SDF with WAI. This may be because the high fibre content enhanced the water-holding capacity of the mushroom powder enriched pasta, which is consistent with the strong positive correlation between moisture content (oven method) and both SI and WAI. Interestingly, significant negative correlations were observed between water content (NIR method) and both SI and WAI, suggesting these techniques measure different characteristics of water with within cooked pasta matrix.

3.2. Near-infrared spectroscopy (NIR)

Table 2 shows the results of NIR analysis, including the calorie,

protein, fat, carbohydrate and water contents of all cooked pasta samples. For white button- and shiitake mushroom powder-supplemented pastas, addition at 10% and 15% increased the caloric contents significantly. In contrast, addition of 5% and 10% porcini mushroom powder reduced the caloric content of the pasta significantly compared with the control. Significant positive correlations were observed between energy and fat contents ($r = 0.902$; $p \leq 0.001$), IDF ($r = 0.755$; $p \leq 0.001$), SDF ($r = 0.695$; $p \leq 0.001$), and TDF ($r = 0.784$; $p \leq 0.001$). Supplementary Table 1 also illustrates negative correlations between energy and carbohydrate, water contents (NIR method) and starch content ($r = -0.611$, -0.721 and -0.811 , respectively; $p \leq 0.001$). Interestingly, there were positive correlations between energy and ORAC ($r = 0.363$, $p \leq 0.05$). Although addition of some mushroom powders increased the caloric content of the pastas, this may be associated with positive health properties in the final products, including increased dietary fibre and antioxidant activity.

3.3. Thermal properties

Table 3 represents the melting enthalpy (ΔH) in all raw (Table 3a) and cooked (Table 3b) pasta samples, and indicates that addition of mushroom powder increased ΔH values compared to control semolina only pasta. This suggests addition of mushroom powders reduced the degree of starch granule gelatinisation or dextrinisation during the both cold extrusion and cooking (Parada, Aguilera, & Brennan, 2011). Supplementary Table 1 shows strong positive correlations between ΔH of raw pasta and IDF, TDF, and fat content, while negative correlation were observed between ΔH of raw pasta and total starch content. However, no significant correlations were observed between ΔH of raw pasta and SDF, or ΔH of cooked pasta and IDF, SDF, TDF, fat and total starch contents. There were positive correlations between ΔH of both raw and cooked pastas and TPC ($r = 0.436$ and 0.734 , respectively; $p \leq 0.05$ and 0.001 , respectively). We speculate that mushroom fibre (especially IDF) and fat had a protective role during processing, reducing the amount of starch gelatinisation: TPC of mushroom powders exhibited same effect on both raw and cooked pasta. This, in turn,

Table 2
The nutritional and energy contents of the control and three species of mushroom-enriched cooked pasta.

Sample name	Energy kcal	Protein g/100 g	Fat g/100 g	Carbohydrate g/100 g	Water g/100 g
CP	377.33 ± 0.58 ^c	6.23 ± 0.23 ^{bc}	2.93 ± 0.12 ^{ef}	81.47 ± 0.31 ^a	3.5 ± 0.17 ^e
5% WBP	377.67 ± 0.58 ^c	7.37 ± 0.40 ^{ab}	2.93 ± 0.058 ^{ef}	80.33 ± 0.31 ^{ab}	4.33 ± 0.058 ^{bcd}
10% WBP	380 ± 0 ^b	6.9 ± 0.56 ^{abc}	3.53 ± 0.29 ^{bc}	80.13 ± 1.14 ^{abc}	4.17 ± 0.47 ^{cde}
15% WBP	383.67 ± 1.15 ^a	7.83 ± 0.57 ^a	4.23 ± 0.15 ^a	78.5 ± 0.44 ^c	3.53 ± 0.31 ^e
5% SP	378 ± 0 ^c	7.13 ± 0.32 ^{ab}	3.4 ± 0 ^{cd}	79.73 ± 0.38 ^{bc}	4.03 ± 0.15 ^{cde}
10% SP	382.33 ± 0.58 ^a	7.13 ± 0.32 ^{ab}	3.87 ± 0.12 ^{ab}	79.63 ± 0.23 ^{bc}	3.73 ± 0.21 ^{de}
15% SP	380.33 ± 0.58 ^b	5.77 ± 0.12 ^c	3.93 ± 0.12 ^a	80.47 ± 0.15 ^{ab}	4.2 ± 0.1 ^{bcdde}
5% PP	374.33 ± 0.58 ^d	6.13 ± 0.32 ^{bc}	3.03 ± 0.058 ^{de}	80.67 ± 0.51 ^{ab}	4.9 ± 0.1 ^{ab}
10% PP	374 ± 0 ^d	7.13 ± 0.31 ^{ab}	2.57 ± 0.058 ^f	80.5 ± 0.53 ^{ab}	5.2 ± 0.26 ^a
15% PP	378.67 ± 1.15 ^{bc}	6.9 ± 0.87 ^{abc}	3.23 ± 0.15 ^{cde}	80.37 ± 1.02 ^{ab}	4.5 ± 0.35 ^{abc}

Mean ± standard deviation. Values within a vertical column followed by the same letter are not significantly different from each other ($p < 0.05$). Abbreviations: CP = control pasta; WBP = white button pasta; SP = shiitake pasta; PP = porcini pasta.

inhibited enzyme accessibility to starch granules within the pasta matrix, limiting the release of reducing sugars during starch digestion. Certainly, [Supplementary Table 1](#) shows the strong negative correlations between ΔH of both raw and cooked pasta, and AUC.

3.4. *In vitro* digestion of cooked pasta

An *in vitro* enzymatic digestion was performed to mimic the behaviour of pasta when eaten. *In vitro* digestibility for the pasta samples are shown in [Fig. 1a–c](#), and represents the amount of reducing sugars released over 120 min *in vitro* digestion. It was noticeable that there was a significant difference between control semolina only pasta and the mushroom powder-supplemented samples. In all samples, values for reducing sugars increased dramatically in the first 20 min and the peak values were reached at either 20 min or 60 min. Significantly more reducing sugars were released from the control pasta than from the mushroom powder-supplemented pastas ([Fig. 1d](#)). The impact of white button, shiitake and porcini mushroom powders in durum wheat semolina pasta on standardised AUC values is shown in [Fig. 1d](#).

Addition of white button and porcini mushroom decreased AUC reducing sugars. However, there were no different differences in AUC amongst the different concentrations (5–15%) of the same mushroom species. For shiitake mushroom powder pasta, AUC values decreased

gradually with increased mushroom powder content, but there were no significant differences between 5% and the control. Substitution of semolina with 15% shiitake mushroom powder was associated with a significant decrease in AUC values. Furthermore, at the same supplementation levels, there was no significant differences in standardised values (except at 5% and 10% shiitake mushroom) for any of the mushroom powder-supplemented pastas.

Previous work had indicated that when co-products from chestnut mushroom were added to extruded snacks they restricted the amount of readily digestible carbohydrates compared with a control sample ([Brennan, Derbyshire, Tiwari, & Brennan, 2012](#)). The rate of carbohydrates digestion in foods controls the glycaemic impact of foods, meaning high GI foods, in which carbohydrate fractions are digested and absorbed rapidly, result in marked fluctuations in blood glucose ([Foschia et al., 2015](#)). The rate and extent of carbohydrate digestion are governed by factors such as structure and composition of starch as well as the amount of fibre, protein and fat within a product ([Dona et al., 2010](#)). For instance, previous research has indicated that a reduction in starch digestibility may be observed by enriching snacks with barley β -glucan (BBG) or mushroom β -glucan ([Brennan, Derbyshire, Tiwari, & Brennan, 2013](#)).

Starch can be classified as rapidly digested starch (RDS), slowly digested starch (SDS) and resistant starch (RS) ([Englyst, Kingman, &](#)

Table 3
Thermal properties (DSC measurements) for raw (a) and cooked pasta (b).

(a)					
	T onset °C	T gelatinization °C	T endset °C	ΔH J/g	ΔTr °C
CP	58.52 ± 1.69	65.02 ± 1.36	68.36 ± 0.79	3.31 ± 0.77	9.85 ± 0.90
5% WBP	56.02 ± 1.24	63.16 ± 2.42	68.35 ± 1.22	2.96 ± 0.40	12.33 ± 0.01
10% WBP	54.29 ± 0.62	62.91 ± 0.62	69.03 ± 0.23	3.97 ± 0.36	14.75 ± 0.84
15% WBP	55.07 ± 0.73	63.8 ± 0.66	71.81 ± 1.39	4.83 ± 0.16	16.75 ± 2.11
5% SP	55.72 ± 0.64	63.39 ± 0.75	69.26 ± 1.09	3.66 ± 0.59	13.55 ± 0.45
10% SP	54.2 ± 1.94	62.8 ± 0.23	69.68 ± 1.43	4.46 ± 0.21	15.48 ± 3.37
15% SP	55.32 ± 0.47	62.04 ± 0.51	70.76 ± 1.32	5.51 ± 1.65	15.44 ± 1.79
5% PP	57.19 ± 0.93	65.57 ± 0.33	69.13 ± 1.03	3.94 ± 0.16	11.94 ± 1.97
10% PP	57.23 ± 2.18	63.59 ± 2.23	69.27 ± 1.58	4.74 ± 0.56	12.04 ± 3.75
15% PP	54.22 ± 1.12	64.58 ± 1.87	70.97 ± 1.39	5.22 ± 0.87	16.76 ± 2.51
(b)					
CP	57.87 ± 0.71	67.9 ± 0.47	72.07 ± 0.50	0.71 ± 0.09	14.2 ± 1.22
5% WBP	56.96 ± 0.57	67.2 ± 0.84	70.91 ± 0.20	0.72 ± 0.02	13.95 ± 0.76
10% WBP	58.15 ± 1.02	65.09 ± 2.14	71.2 ± 0.25	0.83 ± 0.09	13.05 ± 1.27
15% WBP	57.39 ± 0.26	63.01 ± 0.05	67.81 ± 0.99	0.84 ± 0.13	10.43 ± 1.25
5% SP	55.68 ± 0.74	63.31 ± 0.21	70.13 ± 2.19	0.63 ± 0.10	14.45 ± 2.93
10% SP	55.69 ± 0.11	62.37 ± 0.12	69.16 ± 0.40	1.14 ± 0.03	13.48 ± 0.29
15% SP	55.82 ± 0.73	63.68 ± 0.96	71.47 ± 0.71	1.49 ± 0.33	15.66 ± 0.02
5% PP	58.21 ± 0.83	66.83 ± 1.07	70.6 ± 2.00	1.33 ± 0.06	12.40 ± 2.84
10% PP	56.97 ± 1.15	66.23 ± 0.35	71.04 ± 0.38	1.24 ± 0.0016	14.07 ± 0.76
15% PP	56.95 ± 0.69	66.08 ± 0.69	72.01 ± 0.08	2.62 ± 0.25	15.07 ± 0.60

Abbreviations: CP = control pasta; WBP = white button pasta; SP = shiitake pasta; PP = porcini pasta.

Cummings, 1992). The SDS fraction is digested after the RDS (Englyst et al., 1992). As shown in Fig. 1, for all pasta samples, the largest increase in reducing sugars released occurred in the first 20 min (the RDS fraction). From Fig. 1, it is clear that there was a transition in the digestion curves, showing a change in reducing sugar production from RDS to SDS. For white button and shiitake mushroom powder-supplemented pasta, between 20 and 120 min, digestion curves were near horizontal. In contrast, porcini mushroom powder-supplemented pasta generated an upward trend between 20 and 60 min, and the growth trend was much slower than in the first 20 min. Potentially, this indicates differences in SDS contents amongst the three mushroom species, although further work is needed to establish the exact relationship between RDS and SDS and sugar release in these samples.

The mushroom powders provided more dietary fibre than semolina alone. Previous research has illustrated the potential to lower glycaemic response to foods by incorporation of different dietary fibre fractions (Foschia 2015; Foschia et al., 2015). Supplementary Table 1 shows the negative correlations between AUC and IDF ($r = -0.393$; $p \leq 0.05$), SDF ($r = -0.428$; $p \leq 0.05$) and TDF ($r = -0.427$; $p \leq 0.05$). Cleary and Brennan (2006) illustrated that addition of a β -glucan fibre fraction from barley to durum wheat pasta resulted in an attenuation of

reducing sugar release following *in vitro* starch digestion. Such observations may be brought about by changes in the starch-protein matrix of pasta and the high water binding capacity of dietary fibres; both of which affect the physico-chemical properties and digestibility of the pasta. Brennan et al., (2013) integrated the β -glucan fibre-rich fractions from mushrooms to form healthy extruded snacks, illustrating that inclusion of mushroom β -glucan-rich fractions reduced the glycaemic response to samples compared with control samples. The viscosity-modifying effect of dietary fibres has been shown to affect the rate of gastric emptying, transition time, and the intestinal absorption of nutrients, which in turn may lead to a decreased glycaemic response (Chillo et al., 2011). Vitaglione, Lumaga, Stanzione, Scafì, and Fogliano (2009) found a significant decrease in hunger, and increased fullness and satiety after incorporation of β -glucan into bread. While the effect of β -glucan on food viscosity depended on concentration and molecular weight (Wood, Beer, & Butler, 2000; Dikeman & Fahey, 2006), the latter can also influence digestion and physiological properties (Kerckhoffs, Hornstra, & Mensink, 2003; Tester & Somerville, 2003).

Although the addition of fibre can provide many positive effects, such as reducing the glycaemic response to pasta, processing and

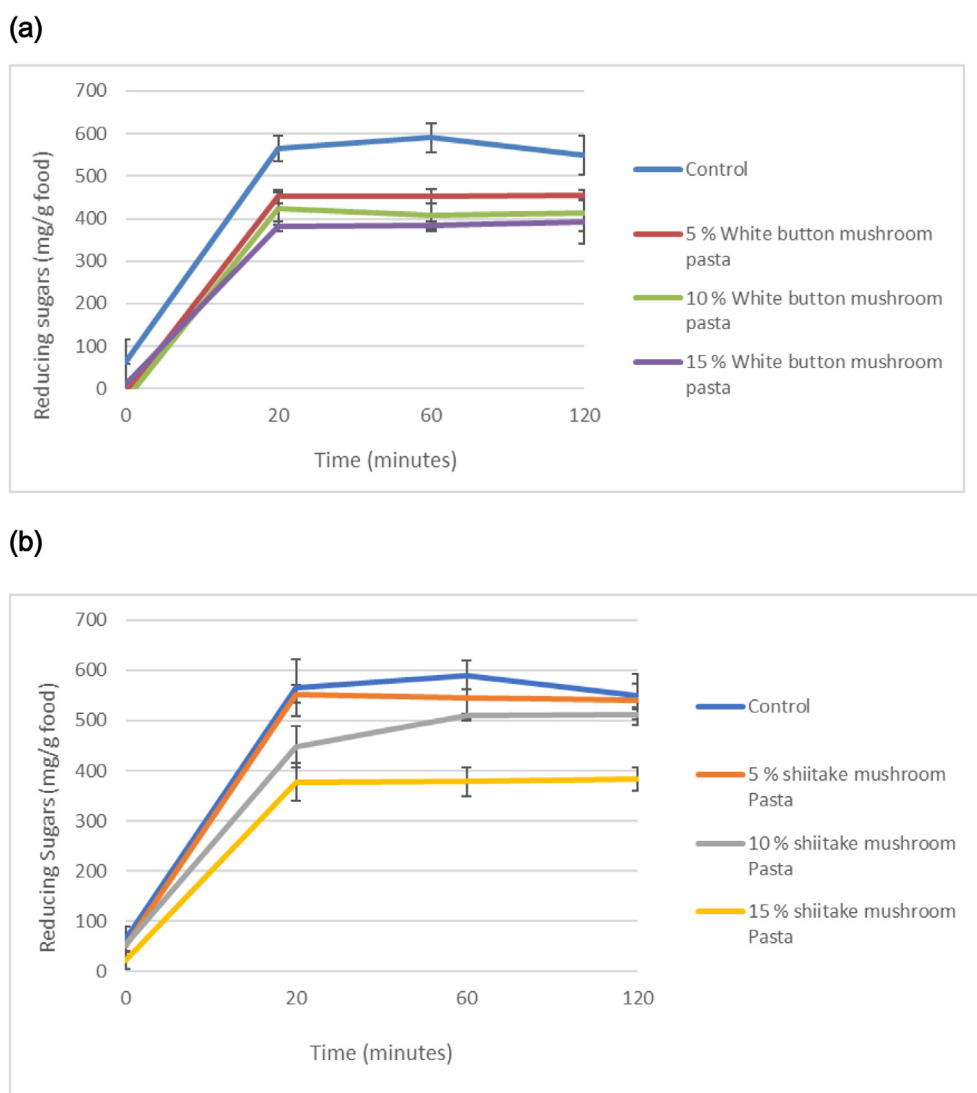


Fig. 1. Standardised AUC; reducing sugars released during *in vitro* digestion. Comparing the control to 5%, 10%, 15% white button mushroom pasta (a); shiitake mushroom pasta (b); and porcini mushroom pasta (c). Values for area under the curve (AUC) (d). Comparing the control to all mushroom powder enriched pasta samples: white button mushroom pasta (WBP); shiitake mushroom pasta (SP) and porcini mushroom pasta (PP). Error bars represent standard deviation of replicates. The same letter is not significantly different from each other ($p < 0.05$).

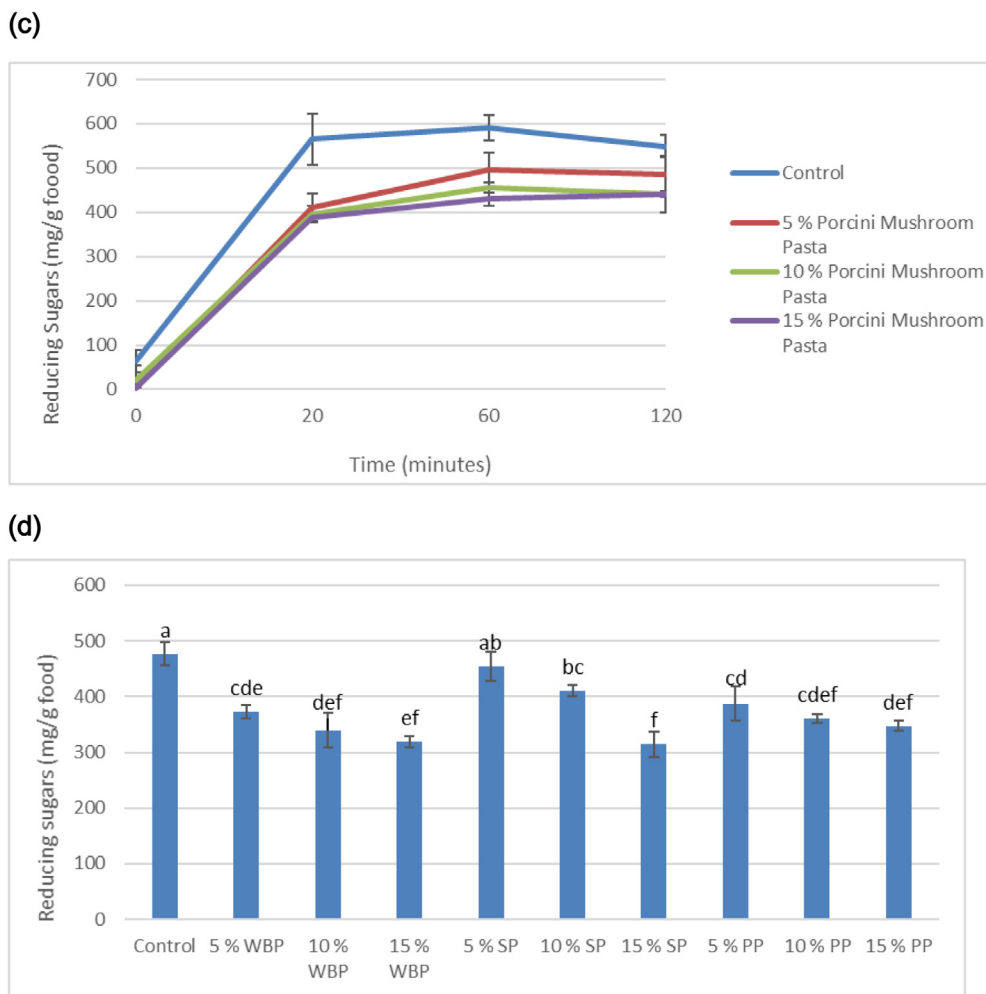


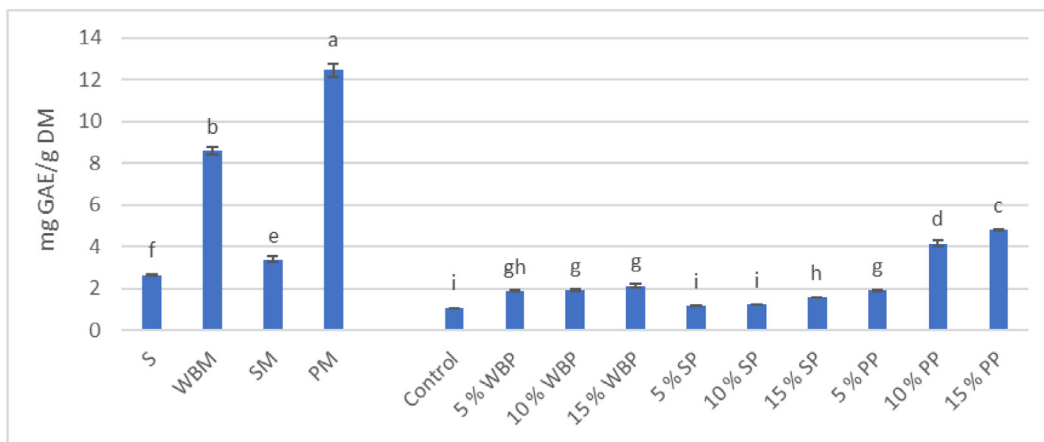
Fig. 1. (continued)

cooking have been shown to influence these properties significantly and the resulting functional benefits (Chillo et al., 2011). The structure of foods has an important part to play in the digestibility of nutrients (Dona et al., 2010). Starch encapsulation by proteins, together with the complexation of starch with lipids and porosity of food structure, are known to limit the extent of starch degradation (Fardet et al., 1999). Cleary and Brennan (2006) proposed that structural modifications made by β -glucan to the protein-starch matrix of pasta were responsible for reduced rates of sugar released during *in vitro* digestion. The protein-starch matrix is, therefore, of considerable importance in terms of pasta structure and function. In this study, the mushroom powders contained more protein than semolina alone, which might contribute to both the nutritional quality and integrity of the protein network in the pastas. Thus, higher levels of protein in the mushroom powder-supplemented pastas may be another explanation for the proportion of starch digested at different time points compared with semolina only control pasta (except for the 5% shiitake mushroom pasta). Previously, it was reported that, the presence of egg white powder in pasta influenced starch digestibility: the larger amounts of protein probably created a stronger network and, thus, reduced the availability of starch granules to digest enzymes (Hager, Czerny, Bez, Zannini, & Arendt, 2013). Similarly, Kim et al. (2008) reported that the presence of starch-protein interactions in pasta dough may be important for reducing the digestibility of starch in pasta and that protein enrichment at a 20% significantly delayed the rate of dextrin release. The effect of protein on starch digestion in pasta could be due to changes in the three-dimensional structure of the protein network as well as potential encapsulation of starch by protein fractions, which reduce enzyme

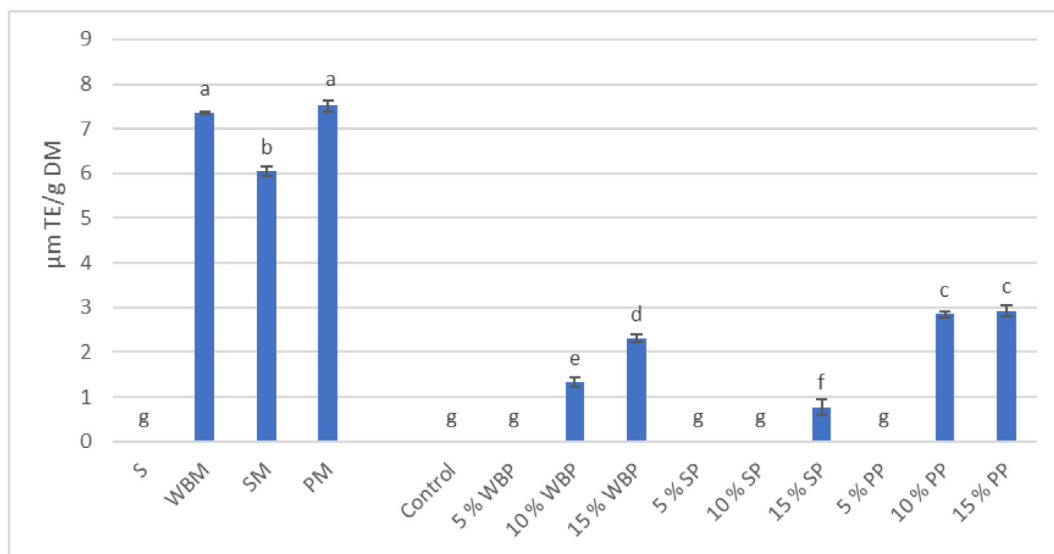
hydrolysis (Foschia 2015; Fardet et al., 1999,1998). Aravind, Sissons, and Fellows (2011) studied durum wheat pasta enriched with purified gluten, gluten, glutenin, gliadin, and low molecular (LMW-GS) or high molecular weight glutenin subunits (HMW-GS) isolated from durum gluten from durum wheat varieties. Inclusion of these protein fractions weakened the dough structure, and the authors proposed that this, in turn, enabled starch granules to become more accessible to starch degrading enzymes, increasing GI values of the protein enriched pastas compared with controls. Further research is under way with the mushroom powder-supplemented pastas to understand more fully these potential mechanisms.

All the mushroom powder pastas contained more fat than semolina only pasta. However, approximately 75% were polyunsaturated fatty acids and included 19.2% palmitic acid, 8.3% oleic acid and 68.8–84% linoleic acid (Cheung, 2008). Previously, it has been illustrated that amylose-lipid complex formation decreased the solubility of amylose, increased gelatinisation temperature, reduced stickiness and freeze-thaw stability, retarded retrogradation, and prolonged storage time (Eliasson, Carlson, & Larsson, 1981; Holm et al., 1983; Krog, 1971). Holm et al. (1983) mixed amylose from potatoes with lysolecithin (palmitic acid) and oleic acid to study the digestibility of amylose-lipid complexes *in vitro*. The study illustrated that complexed amylose affected the rate of starch degradation, due to the formation of a lysolecithin (palmitic)-complex, which could be attributed to a structural disturbance associated with a double bond that rendered the oleic-acid complex more susceptible to α -amylase (Holm et al., 1983). It is possible that, in our research, mushroom powder provided more fat than semolina alone, including palmitic and oleic acids, which could form

(a)



(b)



(c)

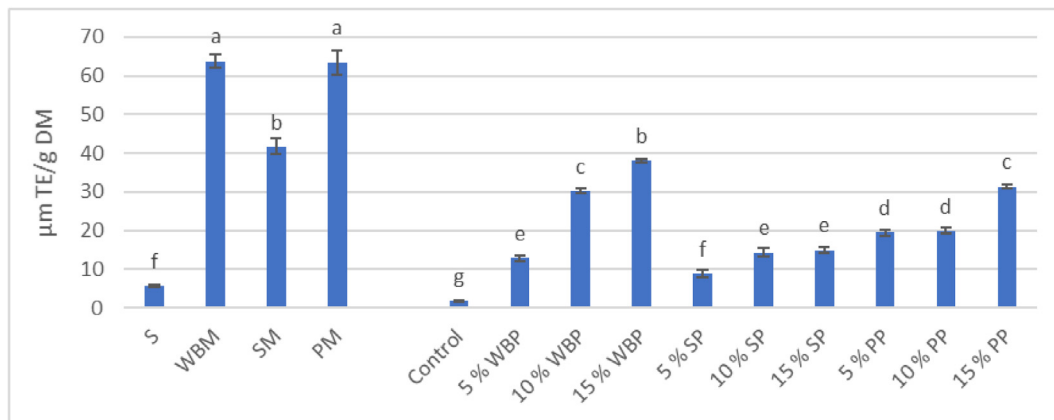


Fig. 2. Values for total phenolic component (TPC) (a) and antioxidant capacities: the DPPH scavenging activities (b) and the ORAC assay results (c). Comparing semolina (S), whole mushroom powder (WBM = white button mushroom; SM = shiitake mushroom; PM = porcini mushroom) and the control sample; white button mushroom pasta (WBP); shiitake mushroom pasta (SP) and porcini mushroom pasta (PP). Error bars represent standard deviation of replicates. The same letter is not significantly different from each other ($p < 0.05$).

such a complex with amylose. This effect could have a role in starch *in vitro* digestibility of mushroom powder-supplemented pasta, and the nature of the lipids in mushroom powder could have an effect on the glycaemic response.

Finally, some components in the mushroom powder (e.g. mushroom fibre) may regulate free and bound water and, hence, water mobility during digestion. Water enzyme mobility and concentration have a role in the effectiveness of starch granule hydrolysis during *in vitro* digestion (Brennan et al., 2012). As reported in previous work (Lu et al., 2016), porcini mushroom powder-supplemented pasta had a smaller swelling index than control pasta. At the molecular level, the low degree of starch swelling, due to restricted water diffusion during cooking, was also believed to be responsible for the slow degradation of starch

(Colonna et al., 1990). We determined a negative correlation between water content (NIR method) and AUC (Supplementary Table 1), which suggests the characteristics of water in mushroom powder-supplemented pastas could also have a role in glycaemic response. In this study, although there was more protein and fat and less starch in mushroom powder-supplemented pasta than semolina pasta, there were no significant correlations observed between AUC and protein, fat and starch contents.

3.5. The antioxidant capacities of semolina and mushroom powder-supplemented pasta samples

Dietary compounds can quench free radicals (antioxidants) in the

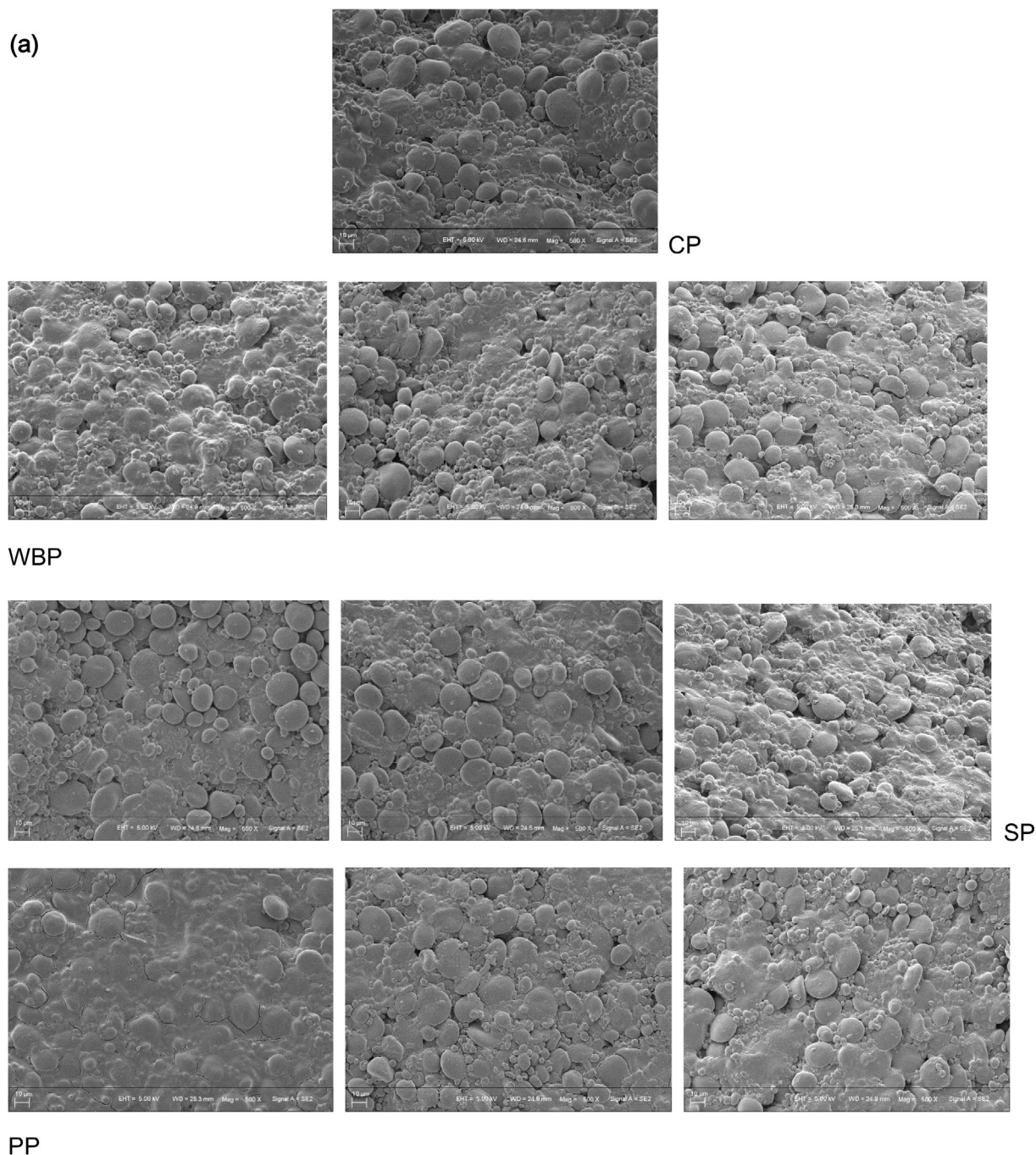


Fig. 3. Scanning electron micrographs of raw pasta longitudinal surface (a) and transverse cross section (b) at 500× magnification: the control pasta (CP); white button mushroom pasta (WBP); shiitake mushroom pasta (SP) and porcini mushroom pasta (PP). From left to right: 5%; 10% and 15% substituent level.

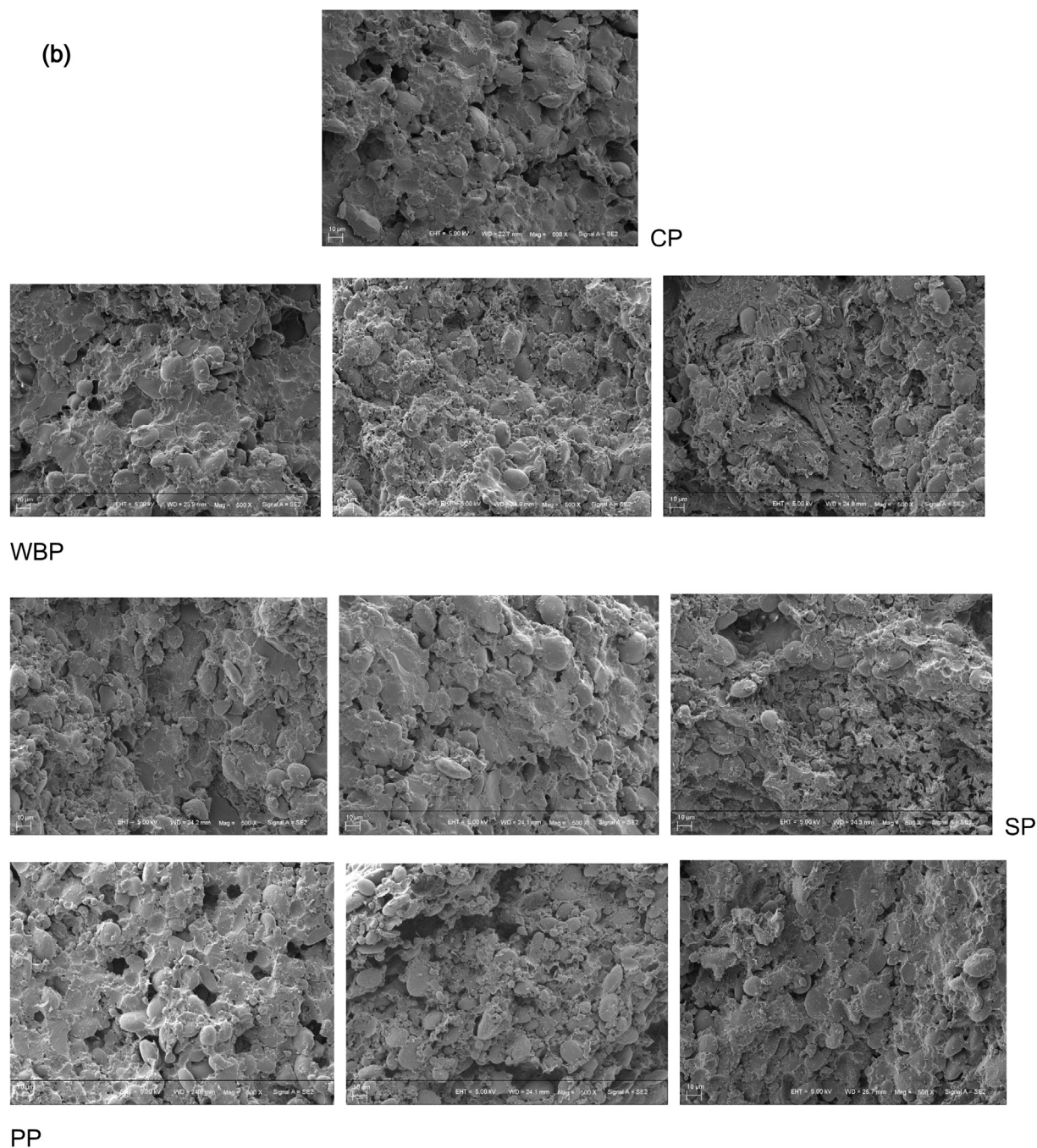


Fig. 3. (continued)

prevention of oxidative stress and have health-promoting effects (bioactive) (Teixeira, Pires, Nunes, & Batista, 2016). The antioxidant properties of foods vary depending on the content of phenolic compounds, vitamins C and E, carotenoids and flavonoids (Saura-Calixto & Goni, 2006). TPC values for the three mushroom-powder-supplemented pastas were significantly higher than semolina only controls: porcini mushroom > white button mushroom > shiitake mushroom > semolina. All the mushroom powder-supplemented pastas had significantly higher values for TPC than control pasta (except 5% and 10% shiitake mushroom pasta). As the content of shiitake and porcini mushroom powders increased, TPC values also increased (Fig. 2a). Fig. 2b and c show that the total antioxidant activity values for semolina, mushroom powders and all pasta samples followed a similar trend. Supplementary Table 1 shows that strong positive correlations existed between TPC, and both DPPH and ORAC ($r = 0.862$ and 0.536 ;

$p \leq 0.001$ and 0.01). ORAC values for the mushroom powder-supplemented pastas were significantly higher than controls. Such observations illustrate the potential of improving radical scavenging activities of pastas through the incorporation of mushroom powders.

In this study, all the pasta samples were cooked in boiling water for 6 min. Previous researchers have reported that boiling water could degrade sensitive polyphenols or enhance extraction of some bound polyphenols from pasta matrices (Fares, et al., 2008). Interestingly, Supplementary Table 1 illustrates significant correlations between ORAC and many other components, except TPC, including IDF ($r = 0.419$; $p \leq 0.05$), SDF ($r = 0.730$; $p \leq 0.001$), TDF ($r = 0.532$; $p \leq 0.01$), protein ($r = 0.367$; $p \leq 0.05$), fat ($r = 0.384$; $p \leq 0.05$) and carbohydrate ($r = -0.495$; $p \leq 0.01$). Supplementary Table 1 shows the significant negative correlations between AUC and TPC ($r = -0.684$; $p \leq 0.001$), DPPH ($r = -0.717$; $p \leq 0.001$) and ORAC

($r = -0.742$; $p \leq 0.001$), suggesting there is a mutual inhibition system between the antioxidant capacity and starch digestion of pasta, which could be supported by the negative correlation between ORAC and starch content ($r = -0.433$; $p \leq 0.05$). These findings point to many components (especially the SDF) in the mushroom powders promoting control of free radicals directly and indirectly, enhancing the antioxidant and bioactive benefits of the final products compared to standard alternatives. Additionally, *in vitro* studies and animal models have shown that antioxidants improve insulin secretion and sensitivity, and improve glucose tolerance and diabetic control (Ihara et al., 2000). TPC and ORAC values indicate that pasta could be a good medium to add antioxidant and bioactive compounds to enhance human nutrition.

3.6. Microstructure

Scanning electron microscopy (SEM) was used to investigate the longitudinal surface (Fig. 3a) and transverse cross sections (Fig. 3b) structure of all raw pasta samples. The longitudinal surface micrographs of mushroom powder-supplemented pasta showed minimal differences compared with control. While there were minor differences between the porcini mushroom pasta and control, there appeared to be an amorphous substance covering starch granules within porcini mushroom pasta matrix with fewer spaces (especially the 5% substituent level). Furthermore, less distinct starch granules could be observed in the porcini mushroom pasta. This suggests a denser protein network in the longitudinal surface of porcini mushroom pasta where more starch granules were encapsulated by protein. The fibre provided by porcini mushroom might have a role in such a microstructure; 5% inulin F-HD enriched spaghetti was reported to obtain a thicker protein matrix compared to control samples (Aravind, Sissons, Fellows, Blazek, & Gilbert et al., 2012).

SEM images of transverse cross sections of mushroom powder-supplemented pasta appeared to be a more irregular and uneven compared with control samples. While protein-starch formation within mushroom powder-supplemented pasta matrix remained uniform. Due to the higher fibre content, filament-like structures were visible in samples with 15% mushroom powder, especially 15% white button mushroom pasta. The additional fibre present in the mushroom powder enriched pasta samples appeared to disturb the gluten matrix. Similarly, SEM from a study incorporating pollard showed that pasta with less 40% replacement were minimally disrupted compared with the control pasta (Aravind et al., 2012).

4. Conclusions

Fresh semolina pasta is a staple starchy food that is nutritional imbalanced. Our research proposes this deficiency might be made up, or pasta enhanced as a functional food, by adding different edible mushrooms. Results were encouraging, as they have revealed that mushroom powder-supplemented pastas could be created containing health-promoting bioactive compounds. Digestion *in vitro* highlighted that addition of mushroom powders (white button, shiitake and porcini mushrooms) to durum wheat semolina could reduce the glycaemic response to the pastas. This work illustrates the potential for using mushroom powders to modulate GI and increase the antioxidant/bioactive contents. At this stage, it would be interesting to undertake further studies of mushroom powder-supplemented pasta using *in vivo* starch digestion analysis and develop more functional pastas containing bioactive substances derived from mushrooms to provide high-quality protein, dietary fibre and polyunsaturated fatty acids for consumers.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.foodchem.2018.04.130>.

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